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ORIGINAL ARTICLE

Phylogenetic analyses of peanut resistance gene candidates and screening of different genotypes for polymorphic markers

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Abstract The nucleotide-binding-site-leucine-rich-repeat (NBS–LRR)-encoding gene family has attracted much research interest because approximately 75% of the plant disease resistance genes that have been cloned to date are from this gene family. Here, we describe a collection of peanut NBS–LRR resistance gene candidates (RGCs) isolated from peanut (*Arachis*) species by mining Gene Bank data base. NBS–LRR sequences assembled into TIR-NBS-LRR (75.4%) and non-TIR-NBS-LRR (24.6%) subfamilies. Total of 20 distinct clades were identified and showed a high level of sequence divergence within TIR-NBS and non-TIR-NBS subfamilies. Thirty-four primer pairs were designed from these RGC sequences and used for screening different genotypes belonging to wild and cultivated peanuts. Therefore, peanut RGC identified in this study will provide useful tools for developing DNA markers and cloning the genes for resistance to different pathogens in peanut.

1. Introduction

The success of a pathogen in infecting a host plant depends on how rapidly the plant recognizes the pathogen and activates appropriate defence reactions. If the pathogen carries an *Avr* (avirulence) gene whose product is specifically recognized by product of the corresponding *R* (resistance) gene in the plant, resistance mechanisms are triggered rapidly, resulting in disease resistance. But, if either the *Avr* or the *R* gene is absent, the pathogen is not recognized rapidly, the defence responses are activated slowly, and disease ensues (Dangl and Jones, 2001, 2006). The largest class of these *R* genes code for proteins nucleotide-binding site (NBS) and leucine-rich repeat (LRR) domains (Hammond-Kosack and Jones, 1997; Hulbert et al., 2001). In the past several years, more than 40 genes

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Table 1 Genotype name, plant introduction number, scientific name and pathogen resistance of different peanut genotypes that were used as a DNA template for polymorphic marker screening.

Genotype	PI number	Scientific name	Resistant to
DUR-25	PI 475887	*Arachis duranensis*	Early leaf spot (*Cercospora arachidis*)
DUR35	PI 497483	*Arachis duranensis*	Rust (*Puccinia arachidis*)
BAT-6	PI 468324	*Arachis batizocoi*	Root-knot nematode (*Meloidogyne javanica*)
BAT-8	PI 468326	*Arachis batizocoi*	Tomato spotted wilt virus
AEQ-2	PI 497630	*A. hypogaea var. aequatoriana*	–
PRV-1	PI 502045	*A. hypogaea var. peruviana*	–
HIR-3	PI 576613	*A. hypogaea var. hirsuta*	–
FST-3	PI 497471	*A. hypogaea var. fastigiata*	–

conferring resistance to different pathogens, including bacteria, fungi, nematodes, and viruses, have been cloned in different plant species. Among the total number of cloned plant disease resistance genes, approximately 75% were from the NBS–LRR gene family (Hulbert et al., 2001). Thus, isolation and characterization of the NBS–LRR-encoding genes are extremely significant for understanding plant–pathogen interactions and development of novel approaches to effective control of plant pathogens in agriculture.

The NBS sequence of *R* genes are characterized by the presence of up to seven conserved domains including the P-loop, Kinase-2 and GLPL motifs (Meyers et al., 1999). The presence of these conserved domains has enabled rapid isolation of resistance gene candidate (RGC) from different plant species by using a polymerase chain reaction (PCR) with degenerate oligonucleotide primers designed from these domains. RGCs were isolated from several plant species, such as cotton (He et al., 2004), potato (Leister et al., 1996), soybean (Yu et al., 1996), lettuce (Shen et al., 1998), tomato (Pan et al., 2000), rice (Mago et al., 1999), barley (Leister et al., 1998), wheat (Seah et al., 2000), chickpea (Huettel et al., 2002), *Medicago truncatula* (Zhu et al., 2002) and Sunflower (Radwan et al., 2003, 2004, 2008).

Peanut or groundnut (*Arachis hypogaea* L.) is the fourth most important oil seed crop in the world, cultivated mainly in tropical, subtropical and warm temperate climates (FAO, 2004). It is an important crop for human food as well as animal feed. However, peanut yields are reduced around the world by fungal, bacterial, viral and root-knot nematode diseases. For example, root-knot nematode disease is causing losses of up to 12% in United States and India (Bailey, 2002). The first significant step for isolating NBS–LRR RGCs from the peanut genome was done by Yuksel et al. (2005) and Bertioli et al. (2003) who used the degenerate primers complementary to highly conserved sequences in the NBS domain.

In this current work, we describe a collection of peanut NBS–LRR resistance gene candidates (RGCs). Phylogenetic analyses were used to estimate the relationships within and among peanut RGCs as well as with cloned plant NBS–LRR-encoding *R* genes. Several genotypes belonging to wild and cultivated peanut were screened by using primers, which were designed from these RGC sequences.

2. Materials and methods

2.1. Plant materials and DNA extraction

Eight peanut genotypes resistant to different types of pathogens; two strains belonging to *Arachis duranesis*, two strains belonging to *A. batizocoi* and four varieties belonging to *A. hypogaea* (Table 1) were used for screening by different primers, which were designed from RGC sequences. Genomic DNAs were isolated from peanut genotype fresh leaf tissues using a modified CTAB (cetyltrimethylammonium bromide) method (Webb and Knapp, 1990).

2.2. Data base searching for peanut RGCs

The database of National Centre for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov) was searched for the RGCs corresponding to NBS–LRR class of disease resistance genes. The keywords 'Peanut resistance gene candidates, Peanut NBS–LRR and Peanut NBS' were used for searching. We have chosen only the RGCs that have NBS domain, and cover the region between the P-loop and the GLPL motif.

2.3. Sequence analyses

The sequences were translated to suitable open reading frame using option 'DNA sequence translation in six-frames' available at http://cgpdb.ucdavis.edu/database/sms/translation.php. The translated sequences were compared with Gene Bank data base using Blast_N algorithms (Altschul et al., 1997). The nucleotide and amino acid sequences were aligned using Clustal_X (Thompson et al., 1997). The redundancy removal was carried out by 'Jalview' available at (http://www.ebi.ac.uk/~michele/jalview/download.html). The phylogenetic tree was constructed using the neighbor-joining method as implemented in Clustal_X with 1000 bootstrap sampling steps (Saitou and Nei, 1987).

2.4. PCR screening of different genotypes

Sequence specific primers (Table 2) were designed based on the sequences of peanut RGCs. The PCR (20 µl total) was carried out using 30 ng of DNA template, 0.65 U Taq polymerase (Qiagen, USA), 1 × PCR reaction buffer, 2.5 mM $MgCl_2$, 0.2 mM of each dNTP and 0.16 µM of each primer. A 'touchdown' PCR protocol was used. The initial denaturation of 94 °C for 3 min was followed by 1 cycle of 94 °C for 30 s, 68 °C for 30 s and 72 °C for 60 s. In each subsequent cycle, the annealing temperature was decreased by 1 °C till reached 58 °C. The amplification continued for 35 cycles at 94 °C for 30 s, 58 °C for 30 s and 72 °C for 60 s. The final extension was carried out at 72 °C for 10 min. After verification of PCR product using 1.5% agarose gel, the single-strand conformational polymorphism (SSCP) was checked for PCR amplicons as described by Slabaugh et al. (1997).

Table 2 Primers name and accession numbers of RGC sequences, which were used to design these primers and the forward and reverse sequences of these primers. A* and B* indicate the polymorphic (Yes) or monomorphic (No) between wild and cultivated and among cultivated cultivars, respectively. CC = coiled-coil and TIR = intracellular effector domain of *Drosophila* Toll and human interleukin-1 receptor.

Primer name	Accession number	TIR/CC-NBS-LRR	Primer (5′- - - - - - - -3′)	A*	B*
P-RGC1	AY157805	CC	**For:** tttgagtgaagaggaggcattt	Yes	No
			Rev: aaacttgagaacaccgaacaca		
P-RGC2	AY157810	CC	**For:** gttgatggagggatttcataaga	No	No
			Rev: ctgacaacgtgttaaggacgag		
P-RGC3	AY157794	CC	**For:** ttgttctggatgatgtttggag	Yes	No
			Rev: cgtaataagcgaccaagtgtttc		
P-RGC4	AY821883	CC	**For:** aggagagagaatgtggtggatg	Yes	Yes
			Rev: gcttcagccttgggtacttct		
P-RGC5	AY157798	CC	**For:** agaattgttgccactgcttctc	Yes	No
			Rev: gaatgtcccatgtgttgcttt		
P-RGC6	AY157777	CC	**For:** gcctgtgtttgggttactgttt	Yes	Yes
			Rev: ccttacccttggattgtatgct		
P-RGC7	AY157799	CC	**For:** ttgtgtgtggattaccgtttct	Yes	Yes
			Rev: ttctacctcagcccaaacttca		
P-RGC8	AY157782	CC	**For:** ttgttgagccttattcggtgtt	No	No
			Rev: aagttcccagctttgttcttca		
P-RGC9	AY157941	TIR	**For:** gtggcagtgtataattggcttgt	Yes	No
			Rev: gcatctaattgctcttgttggtc		
P-RGC10	AY157944	TIR	**For:** aaattcagcaacacacgacca	Yes	No
			Rev: gagcaatccttctaagcaaacaaa		
P-RGC11	AY157943	TIR	**For:** cgaaggtgtatgtttccttggt	Yes	No
			Rev: aggatttccaacaatggcttct		
P-RGC12	AY157781	TIR	**For:** cgcgccgtctacaattcc	Yes	No
			Rev: accagaaccaaaccaaacagag		
P-RGC13	AY157942	TIR	**For:** attcttcgcgttggagaggt	Yes	No
			Rev: tgcttctttgtggaatagctgaa		
P-RGC14	AY157947	TIR	**For:** ttagtccaaggaagggacaaca	Yes	No
			Rev: tgatgaaagagctgaagggatt		
P-RGC15	AY157821	TIR	**For:** ggatgttagggaggtttccaag	Yes	Yes
			Rev: cttcttcttccaccaacccttc		
P-RGC16	AY157780	TIR	**For:** gatgatggaagcctattgaagaa	Yes	No
			Rev: cttaaccacttctgtggacaaatc		
P-RGC17	AY157774	TIR	**For:** tgctagggtcgtgtatggtagaa	Yes	No
			Rev: tcacgccaaggttctctaactg		
P-RGC18	AY747458	CC	**For:** gatcataaggtggaggagcattt	Yes	No
			Rev: ttcgtgtcgggttgttactatg		
P-RGC19	AY747336	CC	**For:** ttgaggcaataacaaagagtcc	Yes	Yes
			Rev: ccaacaatcttcatccgacaa		
P-RGC20	AY747407	CC	**For:** ttgttgtggatgatgtttggag	Yes	No
			Rev: ttctagtgtcgggttcccattt		
P-RGC21	AY747349	CC	**For:** acggtcttgggtttatgtctct	No	No
			Rev: catccggtagatgtgaaaggaa		
P-RGC22	AY747380	TIR	**For:** tcgataatgtggatgatgtgga	Yes	No
			Rev: ttcgcaatgtggaaataacttg		
P-RGC23	AY747362	TIR	**For:** atgatagccggaggacttgatt	Yes	No
			Rev: gggagaccagaagcataagaca		
P-RGC24	AY747331	TIR	**For:** tttgacggtatgtgctttcttg	Yes	No
			Rev: tgccacgaccaaaccaatc		
P-RGC25	AY747352	TIR	**For:** gcttggtttatttccaacgtca	No	No
			Rev: accagtcacatcctcctgctaa		
P-RGC26	AY747555	TIR	**For:** agaagccaattagagaacttagcc	No	No
			Rev: gcggtgagagtgtcgtcttc		
P-RGC27	AY747339	TIR	**For:** gaatttggctgggaatcaagac	Yes	Yes
			Rev: ccgccagtatatttgaccactt		
P-RGC28	AY747508	TIR	**For:** ttagttgctttcttgcggatgt	Yes	No
			Rev: tccatataccctccttcaggttt		
P-RGC29	AY747334	TIR	**For:** agcaagactggtttggttctgg	Yes	No
			Rev: atatttgaccgcctgtttgga		
P-RGC30	AY747415	TIR	**For:** tgaagttgcttgctttcttgct	Yes	No
			Rev: gcaaatgttggtctctggttgt		
P-RGC31	AY747409	TIR	**For:** accattcgatgtggatttgaag	Yes	No

(*continued on next page*)

Table 2 (*continued*)

Primer name	Accession number	TIR/CC-NBS–LRR	Primer (5′- - - - - - - -3′)	A*	B*
P-RGC32	AY747375	TIR	**Rev:** agcgcctctacgtctctagttg **For:** tatgaaggttcctgcttcttgg	Yes	No
P-RGC33	AY747413	TIR	**Rev:** tcttcccattgagcaaatacac **For:** cagtgctattgccgttcaaaga	Yes	Yes
P-RGC34	AY747405	TIR	**Rev:** aactccatgcaagtgctgct **For:** aagactacccttgccaccattt **Rev:** tactccctggacctacccattc	Yes	No

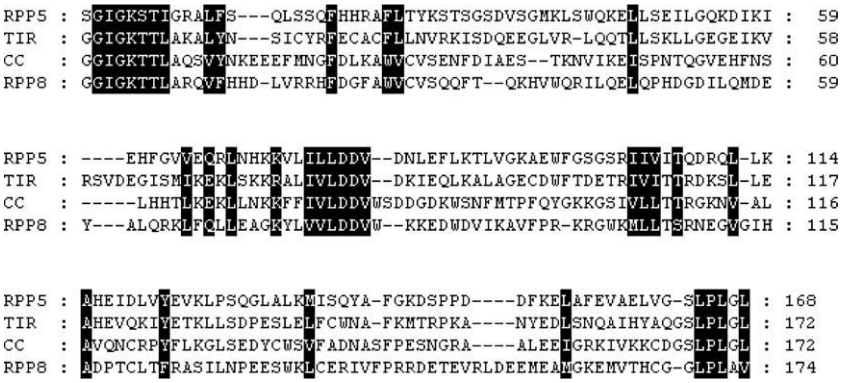

Figure 1 Partial alignment of deduced amino acid sequences of non-TIR-NBS–LRR (CC), TIR-NBS–LRR (TIR) and of two *R*-genes, *A. thaliana RPP8* (Accession No. AAC78631) and *A. thaliana RPP5* (Accession No. NP_849398). The computer program Clustal_X was used in alignment analysis. Alignments were *shaded* using the Genedoc software. The P-loop, Kin-2, Kin-3a (RNBS-B) and GLPL are *underlined*. The RNBS-A of non-TIR-NBS–LRR (FDL × AWVCVSQ × F) and RNBS-A of TIR-NBS–LRR.

3. Results

3.1. Sequence analyses

We have collected a total of 136 RGC sequences from Gene Bank data base, 76 RGC sequences from Bertioli et al. (2003) work and 60 RGC sequences from Yuksel et al. (2005) work. The amino acid identity among these RGCs ranged from 10% to 100%. Most of these sequences have NBS domain, and cover the region between the P-loop and the GLPL motifs. All the expected motifs, including the P-loop, RNBS-A, Kinase-2, Kinase-3a and GLPL, are found in all sequences (Fig. 1). By using the standard differentiation between non-TIR-NBS–LRR and TIR-NBS–LRR sub families (Meyers et al., 1999), two sub families of peanut RGCs could be identified (Fig. 1). The first one, TIR-NBS–LRR, is characterized by the presence RNBS-A TIR (FLENIRE × SKKHGLEHLQKKLLSKLL) and an aspartic acid (D) as the final amino acid in motif Kin-2. On the other hand the presence of RNBS-A non-TIR (FDL × AWVCVSQ × F) and a tryptophan residue (W) as the final amino acid in motif Kin-2 was used to recognize the non-TIR-NBS–LRR subfamily. Seventy-five percent of amino acid sequences were found to be TIR-NBS–LRR, whereas about 25% were non-TIR-NBS–LRR.

3.2. Phylogenetic analyses of peanut RGCs

After eliminating amino acid sequences with >90% identity, a total of 53 RGCs, isolated using degenerate primers (Bertioli et al., 2003; Yuksel et al., 2005) aligned for phylogenetic analyses. A neighbor-joining tree (Fig. 2) constructed from the aligned sequences grouped these sequences into two major branches, comprising TIR and non-TIR subfamilies. The two branches were further supported by the presence or absence of characteristic subfamily-specific consensus sequences, RNBS-A TIR, RNBS-A non-TIR, and Kin-2, consistent with results reported by Meyers et al. (1999).

The distribution of peanut RGCs within Fig. 2 reveals a high level of divergence of sequences within both TIR and non-TIR subfamilies. Within the non-TIR-NBS subfamily, sequences can be subdivided into 10 well-supported major clades, designated as N1–N10. First clade (N1) contains 2 sequences; one of them belongs to peanut RGC1, whereas the other is *RPS5* resistance gene (Accession No. NP_172686) belongs to *Arabidopsis thaliana*, amino acid identity within this clade is 38%. Second clade (N2) contains 2 RGCs (RGC5 and RGC21) and *RP1* resistance gene belongs to *Zea mays* (Accession No. AAP81261) and amino acid identity ranged from 27% to 33%. Of third (N3), sixth (N6) and the eighth (N8) clades, each of them contains only one RGC. Identity within fourth clade (N4) is 44% and it contains two sequences, one belonging to peanut (RGC18) and other belonging to *I2* resistance gene (Accession No. AA27815) of *Lycopersicon esculentum*. Fifth clade contains three sequences that share 45–54% of identity. The amino acid identity within N7 clade ranged from 60% to 89%. Ninth clade contains two (RGC7 and RGC8) belonging to peanut RGCs and *RPP13* resistance gene (Accession No. NP_190237) belonging to *A. thaliana*. The

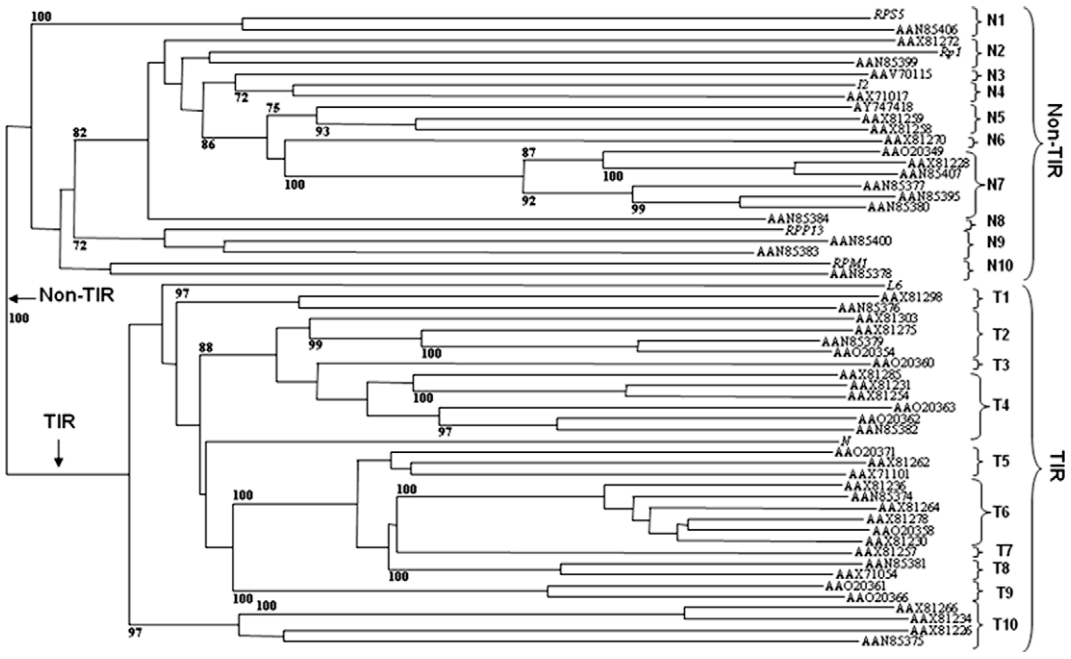

Figure 2 A neighbor-Joining tree of peanut RGCs with 7 cloned *R*-genes encoding NBS–LRR. The tree constructed by Neighbor-Joining method after multiple alignments of polypeptide sequences with CLUSTAL_X (Thompson et al., 1997). Sequences which share more than 90% identity are not included. The confidence levels of nodes were tested by the bootstrapping of 1000 replications, and bootstrap values >70% are indicated on the branches. The sunflower NBS–LRR RGCs are grouped into 20 subfamilies, each being indicated by bold-faced letter N1–N10 for non-TIR-NBS subfamily and by bold-faced letter T1–T10 for TIR-NBS subfamily. The plant *R*-genes representing the NBS–LRR class selected from GenBank are italicized and bold-faced.

amino acid identity within this clade ranged from 30% to 45%. The last clade of non-TIR-NBS–LRR subfamily is N10, which contains only two sequences, one belonging to peanut sequence (RGC6) and other belonging to *A. thaliana* sequence (*RPM1*, Accession No. NP_187360).

On the other hand, TIR-NBS–LRR sequences fall within several strongly supported and typically well-represented clades designated T1–T10. Of first (T1), eighth (T8) and the ninth (T9) clades, each of them contains only two RGCs and the amino acid identity within these clades are 40%, 72% and 71%, respectively. Two clades (T3 and T7), each of them contains only one RGC. The second (T2) and the tenth (T10) clades, each of them contains 6 RGCs and the amino acid identity within these clades ranged from 46% to 79% and 75% to 82%, respectively. The amino acid identity within T5 clade ranged from 55% to 57%, which contains 3 RGCs.

3.3. PCR screening of different genotypes for polymorphic markers

Thirty-four primer pairs have been designed from peanut NBS–LRR RGC sequences and used for screening different genotypes belonging to wild and cultivated peanut (Table 1). These DNA markers were highly polymorphic (78.4%) between the wild and cultivated peanut, whereas they showed low polymorphisms (20.6%) within cultivated peanut. Four polymorphic markers (RGC14, 16, 34 and 38) identified by using agarose gel (INDEL) (Fig. 3D), whereas the other detected by using single-strand conformational polymorphism method (Fig. 3A–C). However, out of 34 primer pairs used for screening different peanut genotypes for polymorphic markers, only 5 RGC markers were monomorphic (RGC2, 8, 21, 25 and 26). The results of this screening were summarized in Table 2.

4. Discussion

The genome sequence of *A. thaliana* and *Oryza sativa* are now essentially complete. These plants have been chosen for large-scale sequencing partly because of their small genomes. Crop plants with large genomes are unlikely to be sequenced in the foreseeable future; therefore one of the challenges for genomics is to use the information available from completely sequenced model plants to further understanding and manipulation of non-model plants. One approach to do that is to identify resistance gene candidates (RGCs) in model plants, and then clone and characterize homologues from non-model plants. In peanut, Bertioli et al. (2003) and Yuksel et al. (2005) used the degenerate primers complementary to highly conserved sequences in the NBS domain of NBS–LRR *R* genes of other species and isolated a total of 136 RGCs. Here, we used these RGCs sequences to create a phylogenetic tree of peanut and design primers for screening different genotypes of wild and cultivated peanut.

The NBS domain is a conservative region between NBS–LRR RGCs and other region as TIR/CC and/or LRR region are the main resources of the variability between *R* genes (Ellis et al., 2000). NBS domain of NBS–LRR genes is preceded by either a coiled-coil (CC) domain or a so-called TIR domain, based on which domain prior to NBS region; three different *R* gene-mediated signaling pathways have been described in *A. thaliana*. The first involves the TIR-NBS-LRR subfamily

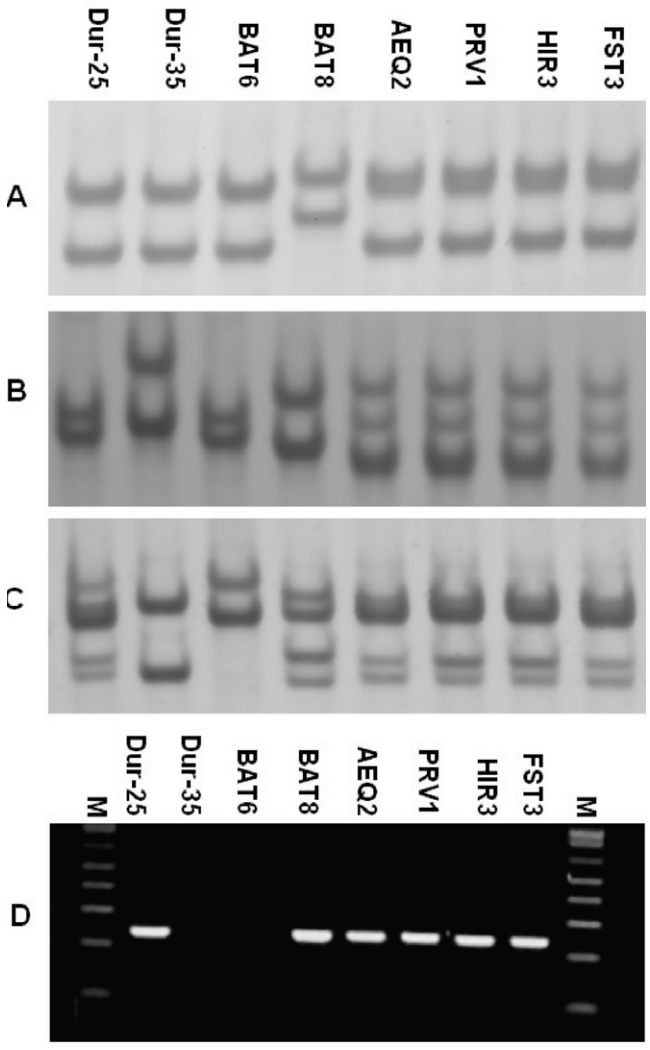

Figure 3 Polymorphic RGC markers detected by SSCP (A, B and C) and INDEL agarose (D) using different wild and cultivated peanut genotypes as DNA templates. A = RGC 18, B = RGC5, C = RGC33 and D = RGC16. M = DNA marker.

of *R* genes (e.g. *RPP1* and *RPP5*) and requires *EDS1* and *PAD4*, the second involves a subfamily of CC-NBS–LRR of *R* genes (e.g. *RPM*1 and *RPS2*) and requires functional *NDR1* and *PBS2* while the third pathway involves the remaining of subfamily CC-NBS–LRR (e.g. *RPP7*, *RPP8* and *RPP13*) and is independent on the function of *EDS1*, *PAD4*, *NDR1* and *PBS2* (McDowell et al., 2000). In this current study TIR-NBS–LRR RGCs are about 75.4%, whereas non-TIR-NBS–LRR RGCs are about 24.6%, this result is agreeing with genome-wide analysis of NBS–LRR-encoding genes in *Arabidopsis* (Meyers et al., 2003) where non-TIR-NBS–LRR genes are about 35% while TIR-NBS–LRR genes are about 65%.

The peanut phylogenetic tree could be categorized into 20 distinct clades based on the identity of their amino acid sequences with a high level of sequences divergence within both TIR-NBS–LRR and non-TIR-NBS–LRR subfamilies. This classification is supported by high bootstrap resembling (Fig. 2). The number of subfamilies is similar to that reported in other species such as soybean (Kanazin et al., 1996; Yu et al., 1996), apple (Lee et al., 2003), *Medicago truncatula* (Zhu et al., 2002), *Arabidopsis* (Meyers et al., 2003), cotton (He et al., 2004) and sunflower (Radwan et al., 2008). The number of subfamilies, which identified in this work, is greater than these identified by Yuksel et al. (2005) and equal the subfamily numbers identified by Bertioli et al. (2003). In this current work we identified more non-TIR-NBS–LRR subfamilies comparing with the two previous works (Bertioli et al., 2003; Yuksel et al., 2005). So this current work may give a complete picture of peanut tree, however it is possible that additional subfamilies may be found when additional RGC clones are sequenced. Ten out of 20 subfamilies each consist of only one or two of the RGC analyzed. This difference may reflect, at least in part, the status of each subfamily in the course of RGC family.

RGC gene family is widely divergent in peanut genome (Fig. 2). This may suggest that peanut RGCs are ancient and evolved rapidly. This hypothesis was supported by the fact that 5 of 10 peanut non-TIR-NBS–LRR-encoding RGC subfamilies were claded with 5 NBS–LRR-encoding *R* genes cloned from diverged monocot and dicot plant species, indicating that the peanut RGC and NBS–LRR-encoding *R* genes may share ancestors. The rapid evolution of peanut NBS–LRR RGC seems to be supported by formation of the diverged subfamilies and the fact that any of TIR-NBS–LRR subfamilies were not claded with any of the NBS–LRR-encoding *R* genes included in the phylogenetic analyses. The source of genetic variation within *R* genes has been the subject of much discussion (Noel et al., 1999; Noir et al., 2001). Different genetic mechanisms have been proposed for the evolution of *R* genes such as recombination, unequal crossing over, gene conversion or point mutations (Michelmore and Meyers, 1998; Ellis et al., 2000).

NBS-encoding RGC have been cloned from several plant species and colocalized with many known resistance genes loci, including those for qualitative and quantitative resistance (Wei et al., 2002; Wang et al., 2001; Collins et al., 1998; Radwan et al., 2007). In this current work we designed 34 primer pairs from the peanut NBS–LRR RGC sequences and used to screen different genotypes belonging to wild and cultivated peanut. These DNA markers were highly polymorphic between wild and cultivar, which reflect the polyploidy difference between wild and cultivated peanut. It is known that peanut wild types belong to diploid genome (AA or BB), whereas cultivated belong to tetrapliod genome (AABB). Therefore, peanut RGC identified in this study will provide useful tools for developing DNA markers and cloning the genes for resistance to different pathogens in peanut. The marker development can be carried out through genetic mapping of these RGCs to the major resistance gene loci in peanut genome and the NBS–LRR-encoding *R* genes can be isolated by positional cloning.

References

Altschul, S.F., Madden, T.L., Schaffer, A.A., Zhang, Z., Miller, W., Lipman, D.J., 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25, 3389–3402.

Bailey, J.E., 2002. Peanut disease management. In: 2002 Peanut Information. North Carolina Coop. Ext. Serv., Raleigh, NC, pp. 71–86.

Bertioli, D.J., Leal-Bertioli, S.C.M., Lion, M.B., Santos, V.L., Pappas Jr, G., Cannon, S.B., Guimarães, P.M., 2003. A large scale analysis of resistance gene homologues in *Arachis*. Mol. Gen. Genomics 270, 34–45.

Collins, N.C., Webb, C.A., Seah, S., Ellis, J.G., Hulbert, S.H., Pryor, A., 1998. The isolation and mapping of disease resistance gene analogs in maize. Mol. Plant Microbe Interact. 11, 968–978.

Dangl, J.L., Jones, J.D.G., 2006. The plant immune system. Nature 444, 323–329.

Dangl, J.L., Jones, J.D.G., 2001. Plant pathogen and integrated defence responses to infection. Nature 411, 826–833.

Ellis, J., Dodds, P., Pryor, T., 2000. The generation of plant disease resistance gene specificities. Trends Plant Sci. 5, 373–379.

FAO, 2004. Statistical Yearbook. http://www.fao.org/statistics/yearbook/vol_1_1/site_en.asp?page = production.

Hammond-kosack, K.E., Jones, J.D.G., 1997. Plant disease resistance genes. Annu. Rev. Plant Physiol. Plant Mol. Biol. 48, 575–607.

He, L., Du, C., Covaleda, L., Xu, Z., Robinson, A.F., Yu, J.Z., Kohel, R.J., Zhang, H.B., 2004. Cloning, characterization, and evolution of the NBS–LRR-encoding resistance gene analogue family in polyploid cotton (*Gossypium hirsutum* L.). Mol. Plant Microbe. Interact. 17, 1234–1241.

Huettel, B., Santra, D., Muehlbauer, J., Kahl, G., 2002. Resistance gene analogues of chickpea (*Cicer arietinum* L.): isolation, genetic mapping and association with a *Fusarium* resistance gene cluster. Theor. Appl. Genet. 105, 479–490.

Hulbert, S.H., Webb, C.A., Smith, S.M., Sun, Q., 2001. Resistance gene complexes: evolution and utilization. Annu. Rev. Phytopathol. 39, 285–312.

Kanazin, V., Marek, L.F., Shoemaker, R.C., 1996. Resistance gene analogs are conserved and clustered in soybean. Proc. Natl. Acad. Sci. USA 93, 1146–1150.

Lee, S.Y., Seo, J.S., Rodriguez-Lanetty, M., 2003. Comprative analysis of superfamilies of NBS-encoding disease resistance gene analogs in cultivated and wild apple species. Mol. Gen. Genomics 269, 101–108.

Leister, D., Ballvora, A., Salamini, F., Gebhardt, C., 1996. A PCR-based approach for isolating pathogen resistance genes from potato with potential for wide application in plants. Nat. Genet. 14, 421–429.

Leister, D., Kurth, J., Laurie, D.A., Yano, M., Sasaki, T., Devos, K., Graner, A., Schulze-Lefert, P., 1998. Rapid reorganization of resistance gene homologues in cereal genomes. Proc. Natl. Acad. Sci. USA 95, 370–375.

Mago, R., Nair, S., Mohan, M., 1999. Resistance gene analogues from rice, cloning, sequencing and mapping. Theor. Appl. Genet. 99, 50–57.

McDowell, J.M., Cuzick, A., Can, C., Beynon, J., Dangl, J.L., Holub, E.B., 2000. Downy mildew (Peronospora parasitica) resistance genes in Arabidopsis vary in functional requirements for NDR1, EDS1, NPR1 and salicylic acid accumulation. Plant J. 22, 523–529.

Meyers, B.C., Dickerman, A.W., Michelmore, R.W., Sivaramakrishnan, S., Sobral, B.W., Young, N.D., 1999. Plant disease resistance genes encode members of an ancient and diverse protein family within the nucleotide-binding superfamily. Plant J. 20, 317–332.

Meyers, B.C., Kozik, A., Griego, A., Kuang, H., Michelomore, R.W., 2003. Genome-wide analysis of NBS–LRR-encoding genes in *Arabidopsis*. Plant Cell 15, 809–834.

Michelmore, R.W., Meyers, B.C., 1998. Clusters of resistance genes in plants evolve by divergent selection and a birth-and-death process. Genome Res. 8, 1113–1130.

Noel, L., Moores, T.L., van Der Biezen, E.A., Parniske, M., Daniels, M.J., Parker, J.E., Jones, J.D., 1999. Pronounced intraspecific haplotype divergence at *RPP5* complex disease resistance locus of *Arabidopsis*. Plant Cell 11, 2099–2112.

Noir, S., Combes, M.C., Anthony, F., Lashermes, P., 2001. Origin. Diversity and evolution of NBS-type disease-resistance gene homologues in coffee trees (*Coffea* L.). Mol. Gent. Genomics 265, 654–662.

Pan, Q., Liu, Y.S., Budai-Hadrian, O., Sela, M., Carmel-Goren, L., Zamir, D., Fluhr, R., 2000. Comparative genetics of nucleotide binding site-leucine rich repeat resistance gene homologues in the genomes of two dicotyledons: tomato and *Arabidopsis*. Genetics 155, 309–322.

Radwan, O., Bouzidi, M.F., Vear, F., Philippon, J., Tourvieille de Labrouhe, D., Nicolas, P., Mouzeyar, S., 2003. Identification of non-TIR-NBS–LRR markers linked to *Pl5*/*Pl8* locus for resistance to downy mildew in sunflower. Theor. Appl. Genet. 106, 1438–1446.

Radwan, O., Bouzidi, M.F., Nicolas, P., Mouzeyar, S., 2004. Development of PCR markers for the *Pl5*/*Pl8* locus for resistance to *Plasmopara halstedii* in sunflower, *Helianthus annuus* L. from complete CC-NBS–LRR sequences. Theor. Appl. Genet. 109, 176–185.

Radwan, O.E., Abratti, G., Heesacker, A.F., Bazzalo, M.E., Zambelli, A., Leon, A.J., Knapp, S.J., 2007. Discovery, mapping, and expression of NBS–LRR genes linked to downy mildew and rust resistance gene clusters in sunflower. In: Plant and Animal Genome Conference, San Diego, CA, USA, January 13–17.

Radwan, O., Gandhi, S., Heesacker, A., Whitaker, B., Taylor, C., Plocik, A., Kesseli, R., Kozik, A., Michelmore, R.W., Knapp, S.J., 2008. Genetic diversity and genomic distribution of homologs encoding NBS–LRR disease resistance proteins in sunflower. Mol. Genet. Genomics 280, 111–125.

Saitou, N., Nei, M., 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4, 406–425.

Seah, S., Spielmeyer, W., Jahier, J., Sivasithamparam, K., Lagudah, E.S., 2000. Resistance gene analogs within an introgressed chromosomal segment derived from *Triticum ventricosum* that confers resistance to nematode and rust pathogens in wheat. Mol. Plant Microbe Interact. 3, 334–341.

Shen, K.A., Keyers, B.C., Islam-Faridi, M.N., Chin, D.B., Stelly, D.M., Michelmore, R.W., 1998. Resistance gene candidate identified by PCR with degenerate oligonucleotide primers map to clusters of resistance genes in lettuce. Mol. Plant Microbe Interact. 11, 815–823.

Slabaugh, M.B., Heustis, Leonard.J., Holloway, J.L., Rosato, C., Hongtrakul, V., Martini, N., Toepfer, R., Voetz, M., Schell, J., Knapp, S.J., 1997. Sequence-based genetic markers for genes and gene families: single-strand conformational polymorphism a for the fatty acid synthesis genes of *Cuphea*. Theor. Appl. Genet. 94, 400–408.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgens, D.G., 1997. The CLUSTAL-X windows interface flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 25, 4876–4882.

Wang, Z., Taramino, G., Yang, D., Liu, G., Tingey, S.V., Miao, G.-H., Wang, G.-L., 2001. Rice ESTs with disease-resistance gene or defense-response gene-like sequences mapped to regions containing major resistance genes or QTLs. Mol. Gen. Genomics 265, 302–310.

Webb, D.M., Knapp, S.J., 1990. DNA extraction from a previously recalcitrant plant genus. Mol. Biol. Rep. 8, 180–185.

Wei, F.S., Wong, R.A., Wise, R.P., 2002. Genome dynamics and evolution of the *Mla* (powdery mildew) resistance locus in barley. Plant Cell 14, 1903–1917.

Yu, Yg, Buss, G.R., Saghai Maroof, M.A., 1996. Isolation of the super family of candidate disease-resistance genes in soybean based on a conserved nucleotide-binding site. Proc. Natl. Acad. Sci. USA 93, 11751–11756.

Yuksel, B., Estill, J.C., Schulze, S.R., Paterson, A.H., 2005. Organization and evolution of resistance gene analogs in peanut. Mol. Gen. Genomics 274, 248–263.

Zhu, H., Cannon, S.B., Young, N.D., Cook, D.R., 2002. Phylogeny and genomic organization of the TIR and non-TIR NBS–LRR resistance gene family in *Medicago truncatula*. Mol. Plant Microbe Interact. 15, 529–539.